Hemoglobin Solution as a Plasma Expander: Effects on Blood Viscosity[1] (35464)

SHUNICHI USAMI, SHU CHIEN, AND MAGNUS I. GREGERSEN[2]

Laboratory of Hemorheology, Department of Physiology, Columbia University College of Physicians and Surgeons, New York, New York 10032

In circulatory shock resulting from blood loss and other causes, the common fundamental disturbance lies in the curtailment of flow through the microcirculation and the ensuing metabolic derangement. The objective of treatment is thus not only a restoration of the lost volume but also an improvement of tissue perfusion (1). Therefore, an ideal plasma substitute should (a) exert a sufficient oncotic pressure to maintain the circulating volume; and (b) cause a reduction of blood viscosity, especially under the near stagnant condition (*i.e.*, near-zero shear rates). The present experiments were performed to test the viscometric effects of the stroma-free hemoglobin solution (2, 3) as a potential expander agent.

Materials and Methods. Hemoglobin solution. The material used in this study is the Hemoglobin Blood Expander (Sterile, Pyrogen Free, Lot. No. 826-8933 (8280), supplied by Abbott Laboratories, Chicago, Ill.). Quantitative analysis of the composition of the supplied solution (Table I) shows that it is hypo-osmotic (250 mOsm/kg of H_2O) to plasma. To avoid the swelling of erythrocytes in such a hypotonic medium, NaCl (powder) was added to render the solution isotonic to plasma (302 mOsm/kg of H_2O). A small amount of human serum albumin (powder, supplied by Blood Research Institute, Jamaica Plain, Mass.) was also dissolved in the solution to prevent the crenation of erythrocytes (4).

Preparation of samples. Fresh blood was drawn from the antecubital vein of healthy human donor into heparinized vacutainer (Becton, Dickinson and Co., Rutherford, N.J.). After centrifugation, the plasma and the buffy coat were removed. A portion of the packed erythrocytes (Sample A) was washed three times with a hemoglobin-free Ringer solution (containing 0.25% albumin), and the remainder (Sample B) was washed three times with the isotonic Hemoglobin Blood Expander (Hb solution). The washed erythrocytes in A and B samples were then resuspended in Ringer and Hb solutions, respectively, and the cell percentage was adjusted to 45 $\pm$ 0.5%. By appropriate combination of the A and B samples, a series of RBC suspensions were prepared with a constant cell percentage (45%) but varying hemoglobin concentration (0 to 6 g%) in the suspending medium. Another series of RBC suspensions were prepared in which the total hemoglobin concentration was kept at 16 g/100 ml, but the proportion of intracellular and extracellular hemoglobin was reciprocally varied.

Hematological determinations. The hemoglobin concentration in the suspending media and the total hemoglobin concentration of the RBC suspensions were determined with the cyanmethemoglobin method (5) using Unopette no. 5887 (Becton, Dickinson and Co., Rutherford, N.J.). Hematocrit values were determined with the use of a microcentrifuge (15,000*g* for 5 min), and the cell percentage (H) was calculated as the product of the hematocrit value and the trapping correction factor 0.99 (6).

Viscometry. Viscometric determinations were made with the use of a coaxial cylinder viscometer, which is modified from the original version of the G.D.M. instrument (7). The operational principle is described else-

[1] This work was supported by DA-49-193-MD-2272, the U.S. Army Medical Research and Development Command, National Heart Institute Research Grant HE-06139 and several private donors.

[2] Deceased, August 26, 1969.

TABLE I. Composition of Hemoglobin Blood Expander.

	Concentration			
	Original	Added	Final	Methods
Electrolytes (mEq/liter)				
Na	114.0	26.0	140.0	—[a]
K	4.6	—	4.6	—[a]
Ca	9.8	—	9.8	—[b]
Mg	1.4	—	1.4	—[b]
Cl	93.0	26.0	119.0	—[c]
HCO_3	18.4	—	18.4	—[d]
Osmolal conc (mOsm/kg of H_2O)	250	52	302	—[e]
Hemoglobin (g/100 ml)	6.85	—	6.85	—[f]
Albumin (g/100 ml)	—	0.25	0.25	

[a] Flame photometer (Instrumentation Lab., Inc., Watertown, Mass.).
[b] Atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.).
[c] Buchler-Cotlove chloridometer (Instrumentation Assoc., N.Y., N.Y.).
[d] Astrup microtonometer (London Co., Copenhagen, Denmark).
[e] Fiske osmometer (Advanced Instrument, Inc., Newton Highlands, Mass.).
[f] Cyanmethemoglobin method (7).

where (8). All measurements were carried out at 37° and over a shear rate range of 52 to 0.052 sec^{-1}.

Results and Discussion. Viscosity of hemoglobin solution. The viscosity of the hemoglobin solution shows no shear rate dependence. With rising hemoglobin concentration the viscosity increases (Fig. 1) and the relation is similar to that reported by others (9, 10). The viscosity of the hemoglobin solutions is comparable to that of serum albumin solutions but lower than that of solutions containing dextran with a similar molecular weight.

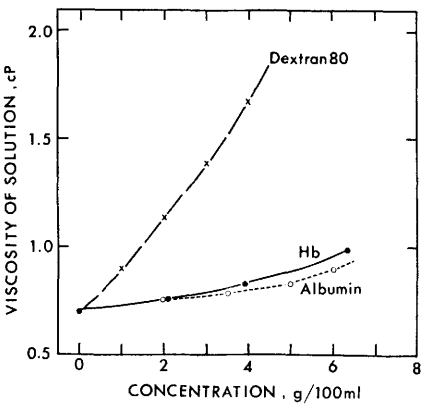

FIG. 1. Relation between viscosity and macromolecular concentration of solutions of dextran 80, hemoglobin, and albumin.

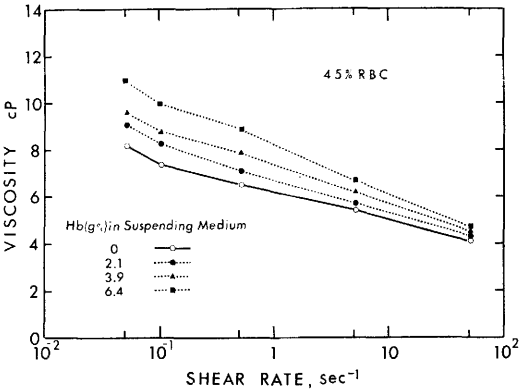

FIG. 2. Effects of hemoglobin solution on viscosity of suspensions of 45% human RBC in Ringer.

Viscosity of 45% RBC suspensions with varying hemoglobin concentrations in the medium. The viscosity of 45% RBC suspensions rises as the shear rate is reduced (Fig. 2). At a constant *H* of 45%, the presence of hemoglobin in the medium causes a slight elevation of viscosity at all shear rates. This increase in viscosity, which is greater at higher hemoglobin concentrations, is comparable to that resulting from albumin, but far less than that seen with other expander agents (*e.g.*, dextran, gelatin, the synthetic polypeptide PAMEG, *etc.*) (11–13). Furthermore, as the increase in viscosity of RBC suspensions in hemoglobin solution is parallel to the rise

in the viscosity of the medium, the relative viscosity (suspension viscosity/medium viscosity) is not significantly elevated from that of RBC in hemoglobin-free Ringer. These findings, as well as the microscopic observation that RBC are monodispersed in hemoglobin solution, indicate that hemoglobin does not interact with RBC to induce cell aggregation.

Viscosity of RBC suspensions with a constant oxygen-carrying capacity but varying hemoglobin concentration in the medium. When the total hemoglobin concentration of the RBC suspensions is kept constant (16 g/100 ml), the viscosity of suspensions in hemoglobin solution is lower than that of the control suspension in Ringer (Fig. 3). The decrease of viscosity parallels the lowering of cell percentage required to achieve a given total hemoglobin concentration. Therefore, the presence of membrane-free hemoglobin reduces the suspension viscosity at a given oxygen-carrying capacity.

General Discussion and Conclusions. The results of the present experiments indicate that the oxygen-carrying capacity of hemoglobin represents a great advantage over other plasma expanders, including albumin. At the same flow rate and hematocrit (Fig. 2), the presence of hemoglobin in plasma would increase oxygen delivery to tissues and alleviate anaerobiosis. At a given oxygen-carrying capacity (Fig. 3), the presence of hemoglobin in plasma reduces the viscosity, and hence oxygen delivery is again increased by the increase in blood flow. In addition, infusion of stroma-free hemoglobin solution does not induce adverse effects on renal function and structure (14, 15). Further experimentation is needed to establish the quantitative aspects of the oxygen dissociation of hemoglobin outside the erythrocytes, especially in view of the important influence of 2,3-diphosphoglyceride and similar agents on oxygen release (16).

Summary. Hemoglobin solution is a promising plasma substitute from a rheological viewpoint. Its addition to blood at a constant hematocrit causes minimum elevation in viscosity but a significant increase in oxygen-carrying capacity. Therefore the administration of hemoglobin may maintain the oxygen-carrying capacity while diluting the red cell concentration, thus facilitating blood flow in the microcirculation and improve tissue metabolism.

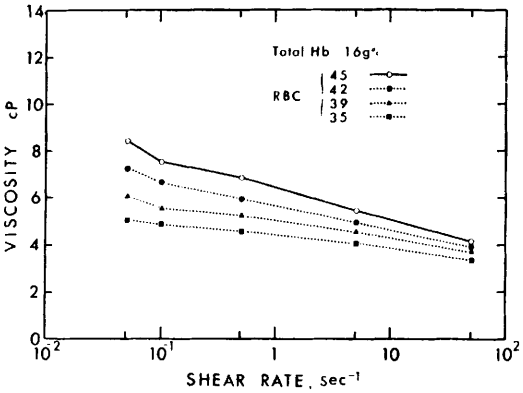

FIG. 3. Viscosity of Ringer suspensions of human RBC with a total hemoglobin concentration of 16 g/100 ml. The cell percentage was varied reciprocally with the extracellular hemoglobin concentration.

1. Gregersen, M. I., *in* "Present status of plasma-expanding agents. Second National Conference on Cardiovascular Diseases" (E. C. Andrus and C. H. Maxwell, eds.), Vol. 1, p. 129. Fed. Amer. Soc. Exp. Biol., Bethesda, Md. (1964).

2. Rabiner, S. F., Helbert, J. R., Lopas, H., and Friedman, L. H., J. Exp. Med. **126**, 1127 (1967).

3. Peskin, G. W., O'Brien, K., and Rabiner, S. F., Surgery **66**, 185 (1969).

4. Ponder, E., "Hemolysis and Related Phenomena." Grune and Stratton, New York (1948).

5. Natelson, S., "Microtechniques of Clinical Chemistry," 229 pp. Thomas, Springfield, Ill. (1961).

6. Chien, S., Dellenback, R. J., Usami, S., and Gregersen, M. I., Proc. Soc. Exp. Biol. Med. **119**, 1155 (1965).

7. Gilinson, P. J., Dauwalter, C. R., and Merrill, E. W., Trans. Soc. Rheol. **7**, 319 (1963).

8. Chien, S., Usami, S., Taylor, H. M., Lundberg, J. L., and Gregersen, M. I., J. Appl. Physiol. **21**, 81 (1966).

9. Cokelet, G. R., and Meiselman, H. J., Science **162**, 275 (1968).

10. Schmidt-Nielsen, K., and Taylor, C. R., Science **162**, 274 (1968).

11. Gregersen, M. I., Usami, S., Peric, B., Chang, C., Sinclair, D., and Chien, S., Biorheology **1**, 247 (1964).

12. Gregersen, M. I., Chien, S., and Usami, S., *in* "Third Conference on Artificial Colloid Agents," p. 29. Nat. Acad. Sci.—Nat. Res. Counc., Washington, D.C. (1965).

13. Chien, S., Usami, S., and Gregersen, M. I., *in* "Body fluid replacement in the surgical patient" (C. L. Fox, Jr., and G. G. Nahas, eds.), p. 138. Grune and Stratton, New York (1970).

14. Peskin, G. W., O'Brien, K., and Rabiner, S. F., Surgery **66**, 185 (1969).

15. Birndorf, N. I., and Lopas, H., J. Appl. Physiol. **29**, 573 (1970).

16. Benesch, R., and Benesch, R. E., Biochem. Biophys. Res. Commun. **26**, 162 (1967).

Received Nov. 11, 1970. P.S.E.B.M., 1971, Vol. 136.